Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 11, pp.60216-60219, November, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANXIOLYTIC EFFECT OF ROOT EXTRACT OF AMAZING FOLKLORE HERB *FLEMINGIA STROBILIFERA* (L.)W.T. AITON IN ALBINO MICE

***[1]Bidhan Mahajon, [2]Rupashri Nath, [3]Santosh Kumar Thakur, [4]Remadevi, R., [5]Ravi Shankar, B.**

[1]Ph.D. Scholar, PG Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur-302002
[2]Ph.D. scholar, PG Department of Roganidana and Vikriti vigyana, National Institute of Ayurveda, Jaipur-302002
[3]P.G Scholar, PG Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur-302002
[4]Ex Professor and HOD, Department of Dravyaguna Vijnanam, Vaidyaratnam P. S. Varier Ayurveda College, Kottakkal, Kerala, India
[5]Director, Department of Pharmacology and Toxicology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, India 574 118

ARTICLE INFO

Article History:
Received 19th August, 2017
Received in revised form
24th September, 2017
Accepted 06th October, 2017
Published online 30th November, 2017

Key words:
Flemingia strobilifera,
Anxiety, Flavonoids, Insomnia.

ABSTRACT

Flemingia strobelifera is a medicinal plant belongs to Fabaceae family. It is widely distributed in India and subcontinent. It is extraordinarily famous for its sleep inducing activity among the tribal people of India. Various studies have already proved its different pharmacological activity. Present study aimed to evaluate the antianxiety activity of aqueous extract of roots of the medicinal plant in elevated zero maze test and mirror chamber test. Twenty four Swiss albino mice of either sex were divided into four different groups of six mice in each. Group I received normal tap water at a dose of 10ml/kg, served as normal control. Group II received Diazepam 2mg/kg and served as reference standard. Group III and IV administered with AEFS at a dose of 200mg/kg and 400mg/kg respectively. In acute toxicity study there was no mortality up to 2000mg/kg. The aqueous extract of the plant has shown significant anxiolytic activity at both the doses. In elevated zero maze test latency period to enter in to open arm has been significantly reduced and there was significant increase in the time spent in open arm and open head dips in comparison to control group (**p<0.01). In mirror chamber test significant increase in the number of partial entries and marked increase in the time spent inside the mirror chamber and number of full entries has observed in comparison to normal control. Presence of Flavonoids might be the chemical entity responsible for its anxiolytic activity. Further scientific studies are required to validate the therapeutic use and to elucidate the exact mechanism of action.

Citation: Bidhan Mahajon, Rupashri Nath, Santosh Kumar Thakur, Remadevi, R., Ravi Shankar, B. 2017. "Anxiolytic effect of root extract of amazing folklore herb *flemingia strobilifera* (l.)w.t. aiton in albino mice", *International Journal of Current Research*, 9, (11), 60216-60219.

INTRODUCTION

Anxiety is a cardinal symptom of many psychiatric disorders and almost inevitable component of many medical and surgical conditions. Currently the benzodiazepines and SSRIs are the most frequently employed medicinal treatments for commonly found clinical anxiety disorders (Feighner JP, 1999; Zohar J, 2003). There are many high potency benzodiazepines such as alprazolam, clonazipam, lorazepam; there is an associated adverse effect like tolerance, dependence and withdrawal symptoms in long term use. Hence there is a continuous search for efficacious and relatively safe drugs having antianxiety potential (Argyropoulos S V *et.al*, 1999; Asnis G M *et al*., 2004; Liebowitz M R, 1993). *Flemingia strobelifera* is a medicinal plant commonly known as *Kamalu* and belongs to Fabaceae family. It is widely distributed in Assam, West Bengal, South India, Andaman and Burma. The plant *Flemingia strobilifera* has been traditionally used in the treatment of epilepsy, insomnia, ulcer, pain and inflammation (Kirtikar K R, Basu B D, 1935; Duthie J S, 1994). Scientific evaluation on *F. strobilifera* has been revealed several pharmacological activity posed by the plant. It has been reportedly exhibited for its different pharmacological activities such as anti microbial, anti epileptic, hepatoprotective, ulcer protective and anti inflammatory activities (Madan S *et al*., 2008; Gahlot K *et al.*, 2013). The phytochemical analysis revealed the presence of flavonoids, glycosides, epoxychromones and pterocarpens (Bhatt S *et al*., 1975;

***Corresponding author: Bidhan Mahajon,**
Ph.D. Scholar, PG Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur-302002.

Saxena VK *et al.*1976; Nigam SS *et al*., 1975; Mahajon Bidhan *et al*., 2014). The present study was aimed to investigate the anti anxiety activity of aqueous extract of root of plant *Flemingia strobilifera* to explore the folklore use of the plant.

MATERIALS AND METHODS

Plant Material

Flemingia strobilifera plants were collected from Jagiroad, Assam during December 2013. It was authenticated by department of Pharmacognosy at SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. A voucher specimen (No. 385/14020702) has been deposited for further future reference.

Preparation of aqueous extract of *F. strobilifera*

The root of plant *F. strobilifera* was shade dried and pulverized, finely sieved and soaked 500g of plant root powder in 2 lit of distilled water for 24 h, after which it was filtered. The filtrate was evaporated in a rotator evaporator and used for the experimentation.

Experimental Animals

Swiss albino mice either sex between 30- 50g body weights were obtained from animal house attached to department of Pharmacology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved by the institutional animal ethical committee under the reference no. SDMCRA/IAEC-2013-14DG 01. The animals were fed with normal rat diet and water *ad libitum* throughout the study period. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12 h light and dark cycle, temperature of 25ºC and relative humidity of approximately 50%.

Acute oral toxicity test

Acute oral toxicity was carried out by following OECD-425 guidelines using AOT software. Albino mice of either sex selected by random sampling were used for acute oral toxicity study. The animal were kept fasting for overnight and provided only with water *ad libitum.* The test drug was administered at a dose of 175mg, 550mg and up to 2000 mg/kg (up and down method). The animals were observed for total 14 days. If any mortality was observed the same dose was repeated again to confirm its toxic potential. If mortality was not observed the procedure was repeated for higher doses in the following order 175, 550 and 2000mg/kg body weight.

MATERIALS AND METHODS

Mirror chamber test

This apparatus is widely used for evaluation of anxiolytic agents belonging to chemically different classes. The apparatus consists of a mirror cube open on one side that is placed inside a squire wooden box. The mirror cube measuring 30cm on a side and an opposite side painted dark brown. The container box is 40X40X 30.5 cm. The mirror cube is generally placed in the centre of the wooden container or box to form a 5cm passage or corridor that completely surrounds the mirror chamber (Reddy DS *et al*., 1997). Animals were placed individually in the chamber of mirrors at a fixed corner and started the stop watch, noted the following parameter for 5 minute- latency of entry, number of partial entries, number of full entries, time spent inside the mirrored chamber during 5 min observation. Increased number of partial and full entries, increased time spent inside the mirrored chamber and shortening of latency to enter the mirror chamber were considered as index of anti-anxiety activity.

Elevated zero maze test

This is a task designed to monitor the level of anxiety. Each mouse is placed on the maze for 5 minutes and the time it spends in the open and closed section is measured. One hour after the administration of the test drugs on 5thday the animals were placed just inside the closed arm.

Table 1. Anti anxiety effect of AEFS on animals in Mirror chamber test

Group	Latency of full entry	Time spent inside the mirror chamber	No. of partial entry	No. of full entry
Control (Group A)	80 ± 44.21	41.5±13.42	9.33±0.98	4.66±1.20
Standard (Group B)	27.83 ± 4.02	45.83±12.28	7.33±1.49	11±0.68*
TED 200mg/kg (Group C)	72.83 ± 28.96	44.16±13.79	11±1.50	6.5±2.72
2TED 400mg/kg (Group D)	28.8±5.598	74.2±17.46	16.8±3.66*	9.8±2.67

DATA: MEAN±SEM **P<0.01,*P<0.05 when comparison to the control group.

Table 2. Anti anxiety effect of AEFS on animals in Zero maze test

Group	Latency Period (sec)	Time spent in open arm	Time spent in closed arm	Open head drops	Closed head drops	Section crossed
Control	39	11.33	247	3.83	21.16	0
(Group-A)	±	±	±	±	±	±
	9.98	4.85	11.49	1.10	0.87	0.00
Standard	17.33	61.83	202.16	17.5	18.16	12.66
(Group-B)	±	±	±	±	±	±
	5.44	13.46**	24.75	3.65*	3.62	1.52**
TED	13	104.66	105.33	21	27	12.16
(Group-C)	±	±	±	±	±	±
	5.07*	10.75**	12.12**	4.40**	2.29	2.02**
2TED	6.5	26.5	210.5	8.83	24.33	0
(Group-D)	±	±	±	±	±	±
	2.21**	9.68	15.22	4.14	2.94	0.00

DATA: MEAN±SEM **P<0.01,*P<0.05 when comparison to the control group.
Abbreviation: AEFS- Aqueous Extract of roots of *Flemingia strobilifera*

The behaviour of the mouse was carefully observed and the following parameters were recorded for the duration of five minutes. The number of entry in to open tunnel, frequency of entry in to open tunnel, number of head dips in the closed

tunnel, number of head dips in the open tunnel and number of times the mouse crossed from one section to the other section of the zero maze were noted down (Kulkarni SK *et al.*, 2007).

RESULTS

The result obtained from the Mirror chamber test indicates that the aqueous extract of *Flemingia strobilifera* (AEFS) has significant anxiolytic activity as compared to the control group. The time spent inside the mirror chamber increased from 41.5±13.42 (sec) in control to 74.2±17.46 (sec) in AEFS at a dose of 400 mg/kg (Table-1). The latency of full entry inside the mirror chamber greatly reduced from 80 ± 44.21 in control to 28.8±5.598 in AEFS at a dose of 400mg/kg (Table-1). There is a significant increase in the number of partial entry in AEFS administered at a dose of 400mg/kg in comparison to control group (*p<0.05). Standard drug (diazepam) has shown significant increase in the number of full entries into the mirror chamber in comparison to control group (*p<0.05). The AEFS at dose 400mg/kg showed increased number of full entry into the mirror chamber and result was comparable with that of diazepam administered group. The result obtained from Elevated zero maze test also clearly shows a significant anxiolytic activity of AEFS as compared to control group. The latency period to enter into the open arm significantly reduced in AEFS administered group at 200mg/kg and 400mg/kg (**p<0.01) in comparison to control group (Table-2). There was a significant increase in the time spent in open arm and number of open head dips in AEFS at 200g/kg in comparison to control group (**p<0.01). The obtained results were comparable with reference standard drug (diazepam) (Table 2).

DISCUSSION

The underlying mechanism or pathology of most of the anxiety disorders is not completely understood. From past research studies has shown the involvement of different ion channels and neurotransmitters such as GABAergic, seratonergic, adrenergic and dopaminergic system in anxiety disorders (Pellow S *et al*., 1985; Kulkarni SK *et al.*, 1996). Currently the benzodiazepines and SSRIs are most commonly employed treatment for the common anxiety disorders. The plant *Flemingia strobilifera* has wide spread traditional use in the treatment of various disorders. There are no available reports on scientific evaluation of its anxiolytic activity. Hence the present study was undertaken to evaluate its anxiolytic activity using Elevated Zero maze test and Mirror chamber test. From the present study it is confirmed that the aqueous extract of root of *Flemingia strobilifera* has strong anxiolytic activity in mice in both elevated zero maze and mirror chamber test (Table-1, 2). Elevated zero maze (Figure-1) is a modification of the elevated plus maze model of anxiety in rodents. It is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA $_A$ – benzodiazepine complex. This task pits the natural curiosity of mice against an inherent fear of new and open exposed places (Kulkarni SK *et al.*, 1996). As mouse overcomes its fear of a new environment their exploratory behaviour increases and they became more willing to fully explore new places. Anxiety like behaviour is assessed by the time and entries into the open relative to enclosed sections of the maze. Drugs that increases open arm exploration, number of open head dips and number of sections crossed are considered as anxiolytics and a greater avoidance of open sections of the maze is used to indicate the heightened levels of anxiety like behaviour.

The present study has observed that the AEFS at 200mg/kg dose produced significant increase in the time spent in the open arm, number of open head dips, and sections crossed. And there is a significant decrease in time spent in the closed arm in the elevated Zero maze model. In mirror chamber test (Figure-2) mice exhibits approach avoidance response upon placement of a mirror within their environment. When a mouse approaches the mirrored chamber it initially doesn't touch the surface but retract to corridor and circles the entire corridor then exhibits a series of partial entries in succession one foot, two feet, three feet and all the four limbs into the mirrored surface. This mirror chamber test is simply to employ non punishing rapid and quantitative and capable of measuring pharmacologically different attribute of anxiety during drug evaluation. The result obtained in the Mirror chamber study showed The AEFS at 200 and 400mg/kg showed marked reduction in the latency to enter into the mirror chamber in comparison to control. The AEFS at 400mg/kg showed significant increase in the number of partial entries and marked increase in the time spent inside the mirrored chamber and number of full entry compared to control. From the earlier studies it has been reported that the plant *Flemingia strobilifera* contains flavonoids, glycosides, epoxychromones and pterocarpens and posses activity against many CNS disorders. Phytochemical analysis of AEFS revealed the presence of flavonoids, saponin, steroids, coumarins, phenol, tannin and terpenoids (S Madana *et al.*, 2008; Kumar A *et al.*, 2011; Swati Madan *et al*., 2013). It might possible that the mechanism of anxiolytic activity of AEFS could be due to binding of these phytochemical to the GABA $_A$-benzodiazepine complex or effect on serotonin transmission or due to its mixed adrenergic potentiation effect for its anxiolytic activity. Also recent study has illustrated the probable *Rasapanchaka* (pharmacological properties in Ayurveda) of the drug as *Rasa-Tikta, Kasaaya* (Bitter and pungent taste)*,Guna-Laghu, Ruksha* (Light and rough virtue)*, Vipaaka-Amla* (Sour metabolic transformation)*, Veerya- Ushna* (Hot active potency) (Mahajon Bidhan *et al*., 2014). Therefore it can be incorporated in Ayurvedic Pharmacopeia of India by gratifying the other indispensable criteria.

Figure 1. Elevated zero maze apparatus

Figure 2. Mirror chamber apparatus

Conclusion

Based on analysis of the result obtained in the present study it can be concluded that aqueous extract of roots of *Flemingia strobilifera* possess significant anxiolytic activity at both the dose levels. And results were comparable with diazepam administered group. The exact mechanism involved is not known. One of the mechanisms involved could be binding of flovanoids to $GABA_A$-benzodiazepine receptor. However further scientific studies are required to confirm the therapeutic. Study suggests that the drug, *F. strobilifera* can be incorporated in Ayurvedic Pharmacopeia of India by satisfying the other obligatory criteria.

Acknowledgement

Authors are highly grateful to Mr. Ravi Munduguru and Mr. Sudhakar, research officers, SDM Centre for research in Ayurveda and allied sciences, Udupi, for their technical support.

REFERENCES

Argyropoulos S V, Nutt D J, 1999. The use of benzodiazepines in anxiety and other disorders. *Eur Neuropsychopharmacol*., 9(6): S 407-12.

Asnis G M., Kohn S R., Henderson M., 2004. Brown N L. SSRIs VS Non-SSRIs in post-traumatic stress disorder: update with recommendations. *Drugs*, 64:383-404.

Bhatt S, 1975. Chalkones and some other constituents of Flemingia strobilifera. *Indian J Chem*., 13:1105-1108.

Duthie J S, 1994. Flora of the upper gangetic plain, Bishen singh Mahendra pal singh Publisher, Dehra doon, pp 215.

Feighner JP, 1999. Overview of antidepressants currently used to treat anxiety disorders. *J Clin Psychiatry.,* 60:18-22.

Gahlot K, Lal VK, Jha S, 2013. Anti convulsant potential of ethanol extracts and their solvent partitioned fractions from Flemingia strobelifera. *Pharmacognosy res.,*5:265.

Kirtikar K R, Basu B D, 1935. Indian Medicinal Plants. vol. 1. Lalit Mohan Basu, Reprint ed., Allahabad, pp 813.

Kulkarni SK, Reddy DS, 1996. Animal behavioural models for testing antianxiety activity. *Meth Find Exp Clin Pharmacol*. 18:219–230.

Kulkarni SK, Singh K, Bishnoi M., 2007. Elevated zero maze: a paradigm to evaluate antianxiety effects of drugs, methods Find. *Exp Clin Pharmacol*., 29 (5):343.

Kumar A, Dora J, Gahlot K, Tripathi R., 2011. Pharmacognostical evaluation of Flemingia strobilifera (R.Br.) root. *IJDDHR*., 1(2): 44-45.

Liebowitz M R, 1993. Depression with anxiety and atypical depression. *J Clin Psychiatry*., 54:10-14.

Madan S., Singh G.N., Kumar Y., Kohli K., Singh R.M.,Mir S.R., Ahmad S., 2008. A New Flavanone from Flemingiastrobilifera (Linn) R. Flemengia antibac, antifungi Br. and its Antimicrobial Activity, *Trop J Pharm Res.*, 7(1): 921-927.

Mahajon B, Remadevi R, Sunil Kumar KN, Ravishankar B., 2014. Preliminary Analysis of Botanical and Phytochemical Features of Kamalu-Root of Flemingia strobilifera (L.) W.T. Aiton. *J Homeop Ayurv Med.,*3:171.

Mahajon Bidhan, B Ravi Shankar, R Remadevi., 2014. Assessment of '*vipaka'* (metabolism) of a new medicinal plant in animal model. *Global J Res Med Plants & Indigen Med.*, 11(3): 427–434.

Nigam SS, Saxena VK, 1975. Isolation and study of the Aurone glycoside Leptosin from the leaves of Flemingia strobilifera. *Planta Med*, 27: 98-100.

Pellow S, Chopin P, File SE, Briley M, 1985. Validation of Open: Closed arm entries in an Elevated Plus-maze as a measure of Anxiety in the Rat. *J Neurosci Methods*., 14:149–167.

Reddy DS, Kulkarni SK, 1997. Differential anxiolytic effects of neurosteroids in the mirrored chamber behaviour test in mice. *Brain Research*.,752: 61-71.

S Madana, GN Singha , Y Kumarb , K Kohlic , R M Singha , SR Mirc and S Ahmad, 2008. A New Flavanone from Flemingia strobilifera (Linn) R. Br. and its Antimicrobial Activity. *Trop J Pharm Res*., 7(1): 921-927.

Saxena VK, 1995. Epoxy chromenes. Therapeutic Agents from *Flemingia strobilifera*. *Asian J Chem.*, 7: 307-310.

Saxena VK, Nigam SS, Singh RB, 1976. Glycosidic principles from the leaves of *Flemingia strobilifera*. *Planta Med*., 29: 94-97.

Swati Madan, Sumeet Gullaiya, Gayendra Nath Singh, Yatin Kumar, 2013. Flemingia strobilifera: Review on Phytochemistry And Pharmacological Aspects. *Int. j. pharmacol*., 4(4):255-262.

Zohar J., 2003. WCA recommendations for the long-term treatment of anxiety disorders. *CNS Spectrums*., 8 (8 Suppl 1):53-61.

****